MUSCLE PROTEIN AND GLYCOGEN RESPONSES TO RECOVERY FROM HYPOGRAVITY AND UNLOADING BY TAIL-CAST SUSPENSION

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Muscle mass, protein, glycogen and tyrosine were measured in hindlimb muscles from rats exposed to 7 days of hypogravity and 12 h of gravity (F) or 6 days of suspension with (R) or without (H) 12 h of loading. Ground control and F rats grew similarly, while H rats grew slower than R or control rats. In F, H and R animals the soleus (SOL) atrophied and the gastrocnemius, plantaris and extensor digitorum longus showed reduced growth. The tibialis anterior showed little response. Changes in mass and protein content correlated in these muscles. Muscles from the F animals showed dramatic increases in glycogen, the SOL being most responsive. H rats showed a greater glycogen concentration in the SOL only, with an even greater value in the R SOL. Only in F SOL was tyrosine greater than the control, suggesting a more negative muscle protein balance, as substantiated in previous studies using H rats. In this study, recovery from suspension decreased SOL tyrosine. These results suggest that the additional stress placed on the F rats post-flight may have prevented the SOL from showing evidence of recovery from hypogravity.

Previous studies in this laboratory using the tail-cast hindlimb suspension model have shown that there are specific changes in protein and carbohydrate metabolism in the soleus muscle due to unloading (1-3). For example, 6 days of unloading caused a 27% decrease in mass and a 60% increase in glycogen content in the soleus muscle, while the extensor digitorum longus muscle was unaffected. Also, fresh tissue tyrosine and its in vitro release from the muscle are increased in the unloaded soleus, indicating that this condition causes a more negative protein balance. With these results in mind, we carried out studies to investigate the effect of hypogravity on protein and carbohydrate metabolism in a number of rat hindlimb muscles.

METHODS

Several groups of animals were studied. One group of 6 rats was flown on the SL-3 mission for 7 days and because of a change in the landing site, was subjected to gravity for 12 h (F). A parallel group of animals was maintained on the ground (G). Because of concerns for the potential effect of 12 h recovery, we ran a subsequent experiment to study this problem. Three groups of animals were used including tail-casted weight bearing controls (C), tail-casted, 6-day suspended, hypokinetic (H) and 6-day suspended followed by 12 h recovery (R).

Muscles from SL-3 rats were dissected, weighed, and frozen in liquid nitrogen by NASA technicians. These were received by us within 36 h of freezing. Upon arrival, the muscles were weighed in a cold room (3°C) and pieces sliced off and placed in either KOH (5N) for glycogen determination or in cold perchloric acid (0.2N) for homogenization. Generally, there was about 15-20 mg muscle/ml acid and about 20-100 mg muscle/ml base. Muscles from rats used in the laboratory were treated in a similar manner. After ethanol precipitation, the glycogen was hydrolyzed in hydrochloric acid (2N at 100°C) and the solution brought to pH 6-8 with KOH (4). Glucose was then assayed enzymatically (5). Homogenates were centrifuged to remove the protein precipitate, and the supernatant was isolated and neutralized to pH 6.5-7.5 with KOH. The protein pellet was redissolved in KOH (1N) and assayed spectrophotometrically (6). The supernatant was analyzed fluorometrically for tyrosine (7) as well as for other amino acids (see this issue, 8).

RESULTS AND DISCUSSION

Overall changes in body weight, and changes in mass, protein, tyrosine, and glycogen in the hindlimb muscles were compared in SL-3 and ground control rats, and also between weight-bearing, hypokinetic, and recovered laboratory animals. As shown in Table 1, there was an identical percent increase in body weight between those rats exposed to hypogravity and ground controls. Similarly, weight-bearing and recovered animals gained weight identically. In contrast, hypokinetic rats experienced a much smaller weight gain. These results indicate that hypogravity does not affect overall body growth as hypokinesia does, and that a 12 h recovery period allows body weight to return to control levels.

Muscle Mass and Protein. The soleus muscle atrophied in the flown, hypokinetic, and recovered groups, while the gastrocnemius, plantaris, and extensor digitorum longus muscles experienced reduced growth. The tibialis anterior showed little response. There appeared to be a good correlation between changes in mass and protein content, as seen in the percent of the expected amount based on normal increases in controls (Table 2). An exception was the recovered soleus, where the mass increased, but the protein decreased. These results indicate that there is a similar response of protein turnover to hypogravity and hypokinesia, with soleus muscle being the most sensitive and the tibialis anterior being almost unresponsive.

Table 1. CHANGES IN BODY WEIGHT

Group	N	Percent of Initial
Ground control	6	+22
Flown	6	+22
Weight bearing	10	+28
Hypokinetic	10	+18
Recovered	10	+28

Table 2. CHANGES IN MUSCLE MASS AND PROTEIN

		Percent of Expected from Controls[a]				
Group	Parameter	SOL	GAS	PLN	EDL	TIB
F	Mass	66	85	84	84	97
	Protein	66	87	83	83	94
H	Mass	71	84	89	85	100
	Protein	69	90	91	92	96
R	Mass	76	86	94	93	98
	Protein	60	87	95	89	93

[a]Amount expected based on normal increases in controls. SOL = soleus, GAS = gastrocnemius, PLN = plantaris, EDL = extensor digitorum longus, TIB = tibialis anterior.

Table 3. CHANGES IN TYROSINE AND GLYCOGEN

		Percent Difference from Controls				
Group	Parameter	SOL	GAS	PLN	EDL	TIB
F	Tyrosine	+37[a]	NS	NS	NS	NS
	Glycogen	+144[d]	+89[d]	+61[a]	+63[a]	+53[a]
H	Tyrosine	NS	NS	NS	NS	+21[a]
	Glycogen	+34[c]	NS	NS	NS	NS
R	Tyrosine	NS	NS	-20[a]	NS	NS
	Glycogen	+63[d]	NS	NS	NS	NS

Abbreviations are as shown in Table 2. Significance of differences: [a]P<0.05, [b]P,0.01, [c]P<0.005, [d]P<0.001. NS = not significant.

Tyrosine. The atrophy and protein loss of the flown soleus was associated with an increase in the tyrosine content of the muscle (Table 3). While the hypokinetic soleus did not display a significant increase in tyrosine in this study, previous work has shown this to occur (2). Among the recovered muscles, only the plantaris appeared to show any significant return to positive protein balance. These results suggest that there is a good correlation between muscle atrophy and tissue tyrosine content.

Glycogen. All muscles analyzed in the flown group showed increases in glycogen concentration, with the soleus experiencing the most dramatic change (Table 3). In contrast, the soleus was the only muscle examined to display any significant changes in glycogen due to hypokinesia, and this increase was even greater in the recovered muscle. These results suggest that while hypokinesia itself causes an increase in glycogen content in the soleus only, in animals exposed to hypogravity there may exist some systemic factor causing glycogen increases in non-atrophying muscles as well. The two factors in tandem may thus bring about the dramatic changes in glycogen concentration seen in the flown soleus.

Concluding Remarks. Despite the fact that the flown animals were exposed to 12 h of normal gravity following 7 days in space, there appears to be a good correlation between changes in muscle mass and protein found in flown and suspended animals. In both groups, the soleus experienced the most severe response, showing a dramatic negative protein balance. This muscle also displayed the most significant increases in glycogen concentration in both groups. Although the additional stress placed on the flown animals during the post-flight period may have altered some metabolic responses, in general the data support the contention that changes in muscle metabolism induced by hypogravity are mimicked by the suspension model (see also this issue, 8).

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